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Fusarium niveum, the Cause of Watermelon Wilt

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Fusarium niveum, the Cause of Watermelon Wilt

By Bailey Sleeth *

WATERMELONS were produced for many years as a profitable crop in the Ohio Valley. About 1915 complaints first were heard that the crop was not doing well, apparently because of some disease. Preliminary investigations disclosed the fact that much of the trouble was due to wilt, a fungous disease caused by *Fusarium niveum* EFS. It was not until 1926, however, that the Station obtained funds to study this problem, and for several years it was largely confined to studies of varieties in an attempt to discover some resistant forms which might be grown profitably in the region. The failure of these early investigations to disclose any short remedy or to find any resistance in commercial sorts of watermelons led to the expansion of the project in 1930. This publication deals with the underlying causes of the failure of resistance under Ohio Valley conditions in the case of those varieties which had been demonstrated as possessing resistance to the disease in other regions.

Watermelon wilt was first reported in 1894 by Smith[15] in South Carolina. He described the causal organism, *Fusarium niveum*, as a "white, vascular-inhabiting fungus." Since that time wilt has been reported from most of the watermelon growing sections of the United States and in many regions it has caused a marked decrease in the watermelon acreage.

Considerable work has been done in an effort to develop a wilt-resistant variety of watermelon. Orton[12,13] was the first to produce such a watermelon, called the Conqueror. However, it was not entirely satisfactory as a commercial type, and its resistance was lost or appreciably decreased when the melon was introduced into new territory, such as Oregon, Iowa, and the Ohio Valley.

The question naturally arises, what was the cause of such a change in the Conqueror watermelon? The following explanations have been proposed: (1) environment, as it affects both host and pathogen; (2) change in the genetic constitution of the suscept; (3) differences in

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physiologic strains of the parasite. While it is probable that all three of these conditions are operative over a period of time and may be intensified by geographic changes, the primary object of the present study was to investigate only the third.

The problem of physiologic specialization has received much attention in the powdery mildews, smuts, and rusts. Much less work has been done with the facultative parasite. However, recent investigations[1,2,9,23,10] have shown that physiologic specialization exists in *Fusarium lini, F. graminearum, F. culmorum, F. avenaceum,* and *F. moniliforme.* It is entirely possible that physiologic strains in the so-called lower types of parasites are as numerous as in the higher parasitic types. Leonian[10] has demonstrated the existence of 110 strains of *Fusarium moniliforme,* a weak pathogen on corn, of which 20 were found to be slightly virulent to corn under greenhouse conditions, 30 exhibited very slight virulence to corn seedlings grown in flasks, and 60 strains were non-virulent under the conditions of the tests. In a preliminary paper the writer[17] reported evidence indicating the existence of physiologic strains of *Fusarium niveum.*

Stakman[21] recognizes physiologic strains by "(1) their pathogenic effect on host plants; (2) morphology, to a limited extent; (3) cultural characters on artificial media; and (4) physico-chemical reactions." According to one or more of these criteria any consistent difference in any two isolants of a given species would be sufficient to establish a physiologic strain or form. While the work herein reported was concerned mainly with the determination of pathogenicity, it has been supplemented with a study of certain cultural characters on artificial media.

THE PATHOGEN

Smith[18] in his first report on watermelon wilt gave the provisional name of *Fusarium niveum* to a wilt-producing, white fungus inhabiting the vascular system of watermelon plants. The following year[19] he described the spore stages as: "(1) minute, elliptical, colorless conidia produced inside the living plant on white mycelium, which plugs the water ducts; (2) large, lunulate, 3- to 5-septate conidia borne on the surface of vines killed by the internal fungus; (3) globose or oblong terminal or intercalary, thin-walled chlamydospores occurring on the surface of the wilted stems."

Despite experimental evidence to the contrary, Smith[20] concluded that the three Fusaria causing wilt of cowpeas, cotton, and watermelon were the imperfect stage of *Neocosmospora vasinfecta* Atk. He classified the Fusarium which caused the watermelon wilt as *Neocosmospora vasinfecta* var. *nivea* (*Fusarium niveum* Erw. Sm.). Later investigations[8,22] have shown that there is no genetic relationship between these parasitic Fusaria and *Neocosmospora vasinfecta.*

The three Fusaria, *F. niveum, F. tracheiphilum,* and *F. vasinfectum,* were considered by Smith[20] to be varieties of the same species, differing only in their host relationship. Orton[12] suggested the analogy of these three organisms to the biological strains of Puccinia and Erysiphe

because of this close morphological resemblance and a common geographical distribution. Later investigators[26] have given specific rank to these three Fusaria, but more recently Butler[3] suggested that these and other Fusaria of the Elegans section should be considered as varieties instead of separate species. Wollenweber[27] has made *F. lycopersici*, *F. blasticola*, *F. tracheiphilum*, *F. batatatis*, and *F. niveum* varieties or forms of *Fusarium bulbigenum* Cooke and Massee.

SOURCE OF MATERIALS

The sources of the isolants used in this investigation are shown in Table 1. Some of the isolants have been in culture for several years, but the greater number were isolated by the writer from diseased plants received from various sources. Single spore cultures of each isolant were obtained by the dilution method and each designated by an Arabic numeral. Although some of the isolants have thrown off numerous dissociants, only a few of these have been continued in culture.

TABLE 1—*Sources of isolants used in investigation*

Insolant	Place of collection	Form of Material	Contributor of material	Received
1	Wood County, W. Va.	Culture	E. C. Sherwood	Jan. 1931
2	Wood County, W. Va.	Culture	E. C. Sherwood	Jan. 1931
3	Wood County, W. Va.	Culture	E. C. Sherwood	Jan. 1931
4	Wood County, W. Va.	Culture	E. C. Sherwood	Jan. 1931
5	Oregon	Culture	E. C. Sherwood	Jan. 1931
6	Expt. Sta. Greenhouse	Melon seedling	Bailey Sleeth	Feb. 1931
7	Expt. Sta. Greenhouse	Melon seedling	A. R. Stanley	Feb. 1931
8	Ames, Iowa	Culture 17	I. J. Wilson	Mar. 1931
9	Ames, Iowa	Culture 14a	I. J. Wilson	Mar. 1931
10	Clemson College, S. C.	Melon vines	G. M. Armstrong	June 1931
11	Clemson College, S. C.	Melon vines	G. M. Armstrong	June 1931
12	Clemson College, S. C.	Melon vines	G. M. Armstrong	June 1931
13	Clemson College, S. C.	Melon vines	G. M. Armstrong	June 1931
14	Lakin, W. Va.	Melon vines	Bailey Sleeth	June 1931
15	Lakin, W. Va.	Melon vines	Bailey Sleeth	June 1931
16	Lakin, W. Va.	Melon vines	Bailey Sleeth	June 1931
17	Lakin, W. Va.	Melon vines	Bailey Sleeth	June 1931
18	Goldsboro, N. C.	Melon vines	S. G. Lehman	July 1931
19	Goldsboro, N. C.	Melon vines	S. G. Lehman	July 1931
20	Goldsboro, N. C.	Melon vines	S. G. Lehman	July 1931
21	Goldsboro, N. C.	Melon vines	S. G. Lehman	July 1931
22	Wellington, Texas	Melon vines	J. J. Taubenhaus	July 1931
23	Wellington, Texas	Melon vines	J. J. Taubenhaus	July 1931

Several varieties of watermelons were used in making the pathogenicity tests. Three of them were of the standard commercial sorts—Gray Monarch, Kleckley Sweet, and Fordhook. Seeds of these varieties were obtained from Ferry-Morse Seed Company, Detroit, and Jerome B. Rice Seed Company, Cambridge, N. Y. Three other varieties, Pride of Muscatine, Iowa Belle, and Iowa King, which have been included in the tests, were bred and selected for wilt resistance in Iowa. These were furnished by the Iowa Melon Growers' Association, Ames, Iowa. In one series of tests the writer used varieties and hybrids furnished by the department of agronomy of the West Virginia Agricultural Experiment Station.

PATHOGENICITY

Soil preparation and infestation: The different isolants of *F. niveum* were tested in the greenhouse on watermelon seedlings over a period of 12 months. The plants were grown in new flats 6 x 16 x 20 inches, made of cypress lumber and filled with soil to within one inch of the top. The soil consisted of a mixture of well-rotted compost and sand autoclaved for two hours at 15 pounds pressure. Before being filled the flats were disinfected by washing with a 1:1000 solution of $HgCl_2$.

The inoculum used to infest the soil was obtained by growing the fungus on rice cultures which were made up by adding 25 gm. rice to 75 cc. of distilled water in liter flasks. The rice was cooked by autoclaving for 30 minutes at 15 pounds pressure, then inoculated and incubated for 28 days at laboratory temperature. At the end of this period the entire mass of rice was permeated with the fungus. The contents of one flask containing a given isolant were thoroughly mixed with the soil of one flat, and each flat was designated by the number of the isolant with which it was inoculated. Twenty-three flats were thus prepared with the various strains. Two flats which received no inoculum were used as checks.

Method of planting: Several series of tests were made with one or more varieties of watermelons in each test. Usually 80 to 100 seeds were planted to a flat. Before planting, all seeds were treated for one hour in a 0.25 percent solution of Semesan, an organic mercury compound, then washed in water and placed in a moist chamber. Twenty-four to 48 hours later seeds of uniform germination were removed and planted three-fourths to one inch deep in the flats.

Symptoms of the disease in the greenhouse: The observed symptoms of seedling infections in the greenhouse were similar to those described by Porter.[14,15,16] These are briefly given below.

Seedling rot was observed and identified as such in a few instances. It is a typical rot of the hypocotyl, killing the seedling shortly after germination and before it emerges from the soil. The cotyledons become infected under ground if the seed germinates sufficiently to free them. Infection takes place through the epidermis, causing necrosis of the external tissues; the fungus thus enters the vascular system and kills the seedling before the latter emerges. Porter[15] observed like symptoms in un-emerged watermelon seedlings. It seems likely that this condition is of importance only in heavily infested soil. However, poor emergence in the field might be due to either seedling rot or poor germination. In some cases, therefore, it is difficult to determine which factor is responsible for the poor stand.

Under certain conditions, such as heavy soil infestation and a warm, moist atmosphere, *damping-off* occurred in a number of the seedlings. Smith[19] found that *F. niveum* attacked all of the tissues, and the plants damped-off in hot, wet weather in large numbers in the field. Porter [15] describes damping-off as occurring at the ground line

where the hypocotyl first becomes water-soaked, "following which the epidermal and cortical tissues become invaded and weakened sufficiently to cause the plant to fall over."

A number of seedlings growing in infected flats were found to be stunted, but otherwise normal. This symptom has been described[15] as *dwarfing* or *stunting;* the plants seem unthrifty, as though sufficient plant food were not available, yet they do not show signs of wilting. The stunting becomes more pronounced as the plants grow older, being manifested by shortened internodes and smaller leaves. Similar symptoms due to other Fusaria have been observed in cotton,[5] tomatoes,[4] and flax.[1]

TABLE 2—*Results of pathogenic tests with various isolants of Fusarium niveum* *

Isolant	Number of plants	Number of plants wilted	Percentage of plants wilted
1	396	353	89
2	311	143	46
3	287	276	96
4	392	345	88
5	330	122	37
6	307	273	89
7	274	127	45
8	309	272	88
9	272	62	23
10	335	7	2
11	297	291	98
12	278	8	3
13	328	0	0
14	322	0	0
15	321	0	0
16	320	266	83
17	315	3	1
18	302	0	0
19	326	0	0
20	331	331	100
21	297	0	0
22	320	0	0
23	321	247	77
Check	315	0	0

* Plants approximately 21 days old and grown in infested flats in the greenhouse.

Wilting of the cotyledons and leaves was the most common symptom of seedling infection observed. Even before wilting was evident to the eye, a slight flaccidity in the infected seedlings could be detected by drawing the finger lightly across the lower surface of the cotyledon. Smith[18] described the symptoms as "those of a plant transpiring freely and insufficiently supplied with water, although at the same time there is an abundance of moisture in the soil."

The browning of the vascular bundles by vascular parasites has been reported frequently. In the present study *discoloration and browning of the vascular bundles* was observed to be related to infection.

Results: Data on infected seedlings are based principally upon wilting of the cotyledons and leaves. However, a small number of damped-off and vascular-discolored plants are included under plants wilted. Since isolations have shown that there is a high degree of cor-

relation between these symptoms and infection by the pathogen, the resulting data are considered to be reliable.

The various isolants were found to differ in virulence, ranging from apparently non-pathogenic to highly-virulent strains. This condition is clearly shown in Table 2. Approximately 21 days after planting, a number of the isolants produced no wilting, while isolants 3, 11, and 20 wilted 96 percent or more of the young plants. These differences in the virulence of the several isolants indicate the presence of different pathogenic strains of the organism.

TABLE 3—*Virulence of 23 isolants, expressed in percentage of plants wilted, based upon time required for isolant 20 to produce 100% wilting in a single variety of a series in greenhouse tests.*

	Test series and varieties								
	I	II		III	IV	V			
	Gray Monarch	Pride of Muscatine	Gray Monarch	Iowa King	Kleckley Sweet	E. Ford-hook	2-4-5 x R	Russian	2-4-7 x EF.
Date of planting	9/10	11/16		12/18	2/8	3/21			
Days after planting	13	18	18	24	28	16	16	16	16
Isolants	Percentage of plants wilted								
1	94	61	86	89	100	26	31	13	67
2	30	32	60	23	37	0	0	0	9
3	100	31	70	90	100	73	77	36	100
4	95	13	50	70	100	52	23	13	83
5	46	0	0	9	40	4	0	0	32
6	90	40	30	74	100	74	44	0	96
7	24	20	35	8	38	14	0	0	29
8	71	30	78	86	100	22	0	6	100
9	0	0	0	7	30	0	0	0	0
10	0	0	0	0	0	0	0	0	0
11	100	100	100	92	100	92	80	25	100
12	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0
16	39	50	83	85	90	13	0	0	83
17	0	0	0	0	0	0	0	0	12
18	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0
20	100	100	100	100	100	100	53	36	100
21	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0
23	87	2	59	70	84	100	15	8	76
Check	0	0	0	0	0	0	0	0	0

In order better to compare the isolants according to their relative virulence, it was found convenient to use strain 20 as a standard. This strain was selected because it was the most virulent of the strains tested, and also because of its apparent stability in artificial culture. Table 3 gives the results of this method of comparison in five series of tests. The table was calculated upon the minimum time necessary for strain 20 to produce symptoms of wilting in all the plants of a single variety within a series.

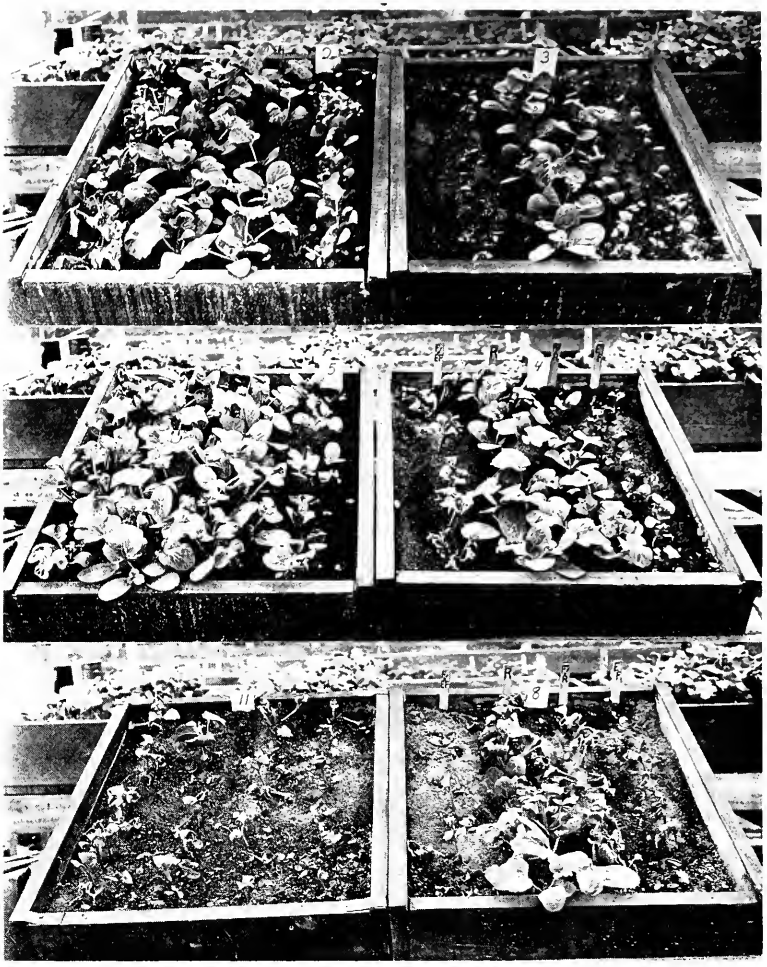

FIG. 1—Watermelon seedlings 21 days after planting from series V. Varieties, left to right, 2-4-7 x EF., Russian, 2-4-5 x R., and Early Fordhook. Top row, *Fusarium niveum* strains 2 and 3; middle row, strains 5 and 4; lower row, strains 11 and 8. Note difference in virulence of the various strains

With one exception, variety 2-4-5 x R., in series V, strain 20 produced the greatest amount of wilt in a given period of time; strain 11 ranked second, and strain 3, third. However, in some instances strains 3 and 11 were more virulent in the early part of the experiment than strain 20 (Table 3). These three strains were consistently pathogenic and highly virulent. Strains 1, 2, 4, 5, 6, 7, 8, 16, and 23 were less

virulent and produced varying amounts of wilt in the same length of time. Two strains, 9 and 17, appeared to be only slightly pathogenic in one or two instances, while the remaining nine failed to produce any visible evidence of wilt within the time required for strain 20 to produce 100 percent wilting. However, these less virulent strains were able to infect the plants as evidenced by their being re-isolated from seedlings. (See Fig. 1.)

A summation of the total amount of wilt produced by the 23 isolants in approximately 21 days in the different varieties of watermelons tested is found in Table 2. These data are in close agreement with those of Table 3, showing that the isolants range from highly-virulent to non-virulent strains.

Some effects of environmental conditions: The time required to produce equivalent amounts of seedling growth and wilt in the pathogenicity tests was influenced by sunlight, humidity, soil moisture, and temperature of the air and soil. The conditions most favorable for the growth of the melon seedlings were apparently the most favorable for

TABLE 4—*Relative virulence of seven strains of Fusarium niveum in greenhouse test, expressed in percentage of plants wilted (series V)*

Isolant	Variety	Number of Plants	Percentage of plants wilting					
			Days after planting					
			10	14	16	19	22	26
1	E. F.	23	0	21	26	61	83	100
	245R	16	0	13	31	50	62	88
	R	16	0	13	13	13	13	50
	247EF	27	4	59	67	85	93	100
3	EF	19	26	74	79	84	89	100
	245R	18	11	72	77	77	83	100
	R	22	23	32	36	36	36	100
	247EF	27	93	100	100	100	100	100
4	EF	17	0	35	52	76	82	100
	245R	13	0	15	23	38	38	100
	R	16	0	13	13	13	13	75
	247EF	24	33	79	83	100	100	100
5	EF	23	0	0	4	17	65	100
	245R	22	0	0	0	0	5	36
	R	29	0	0	0	0	0	21
	247EF	28	0	14	32	50	71	100
8	EF	18	11	16	22	56	100	100
	245R	21	0	0	0	38	86	100
	R	18	0	0	6	16	22	100
	247EF	22	9	82	100	100	100	100
11	EF	24	8	83	92	100	100	100
	245R	15	20	80	80	93	100	100
	R	12	25	25	25	83	92	100
	247EF	24	33	83	100	100	100	100
20	EF	19	0	79	100	100	100	100
	245R	21	5	29	52	90	90	100
	R	17	0	18	36	88	94	100
	247EF	27	33	96	100	100	100	100

* Note that strains 3 and 11 are more virulent than strain 20 on the 10th and 14th days; later strain 20 shows the greater virulence.

infection. This parallel condition is shown by the results obtained in series I and IV, Table 3. Approximately as good growth and an equal amount of infection took place in series I in 13 days after planting as

occurred in series IV in 28 days. The difference in light and temperature available at the time of the tests are considered to be the main contributing factors for this variation, as series I was grown in early September and series IV in early February.

The length of time required to produce the first symptoms of infection varied also with the different strains. Some of the strains, such as 3, 11, and 20 (see Table 4), proved to be highly virulent, causing a high percentage of seedling wilt in a few days, while slightly less virulent strains, such as 1, 4, and 8, required a few days longer. Strains 2, 5, and 7 were much slower in producing symptoms and generally failed to produce as high a percentage of wilt by the end of the test as strains 3, 11, and 20. Strains of low pathogenicity were much slower in producing symptoms, and the non-pathogenic strains failed to produce visible wilting within the duration of the tests.

This difference in virulence of the strains as represented by time required to produce wilt is further illustrated in Table 4 in the case of the Russian variety, an inedible watermelon. At the end of 16 days, strains 3 and 20 had produced 36 percent wilting; strain 4, 13 percent; strain 5, no wilt. Ten days later strains 3 and 20 had produced 100 percent wilt and strains 4 and 5, 75 and 21 percent wilting, respectively. Similar and even more pronounced differences were found in Early Fordhook and 2-4-7 x EF., a hybrid melon.

All the varieties and hybrids tested were found to be somewhat susceptible to the more virulent strains of the wilt organism (see Table 3), while on the other hand the Russian variety appeared to be immune to the less virulent strains.

RELATION OF TEMPERATURE TO GROWTH IN CULTURE

The effect of temperature on the vegetative growth of different Fusarium species on artificial media has been studied and reported by various workers.[6, 4, 25, 23] In general the minimum temperature for growth under laboratory conditions ranges from 5 to 10° C., the maximum from 33 to 37° C., and the optimum from 22 to 30° C. Porter[15] gives the optimum temperature for *Fusarium niveum* to be between 24 and 32° C., with a very slow growth at 12 and 35° C. These results are in agreement with those obtained in the present investigation.

The purpose of the temperature experiments was to determine if there were any distinguishing differences in the minimum, optimum, and maximum temperatures for vegetative growth of the various isolants, as any marked difference in the response to temperature change would prove a useful differential test.

Petri dishes containing 50 cc. of ammonium nitrate agar (NH_4NO_3, 2 g., $MgSO_4$, 1 g., KH_2PO_4, 1 g., dextrose, 10 g., agar, 20 g., and distilled water, 1000 cc.) were inoculated with a circular disk of mycelium 8 mm. in diameter. Care was exercised to keep the pieces of inoculum the same size and to cut them from the same relative position near the margin of

the culture. The substrate of both the old and new cultures was prepared according to the same formula. All cultures were made in duplicates, and the average diameter of the two colonies was recorded.

The cultures were kept in controlled temperature chambers of 15, 20, 25, 30, and 35° C. The measurements of the size of colony, reported in Table 5, were taken seven days after inoculation. The cultures in Figure 2 give some idea concerning the amount of growth of the various isolants under similar conditions.

The optimum temperature range for growth was found to be between 25 and 30° C., the maximum above 35° C., and the minimum, as indicated by preliminary experiments, around 5° C. Distinct differences were manifested by many of the isolants in their growth responses at the different temperatures. The 23 isolants and the 4 dissociants have tentatively been placed in 6 groups, each group containing those which are more nearly alike in growth response. These groups are indicated as follows: group a., containing isolants 1, 2, 3, 5, and 6; group b., 7, 7b, 7c, 10, 12, 13, 14, 15, 17, 18, 19, 22, and 22b; group c., 8, 11c, 16, and 16b; group d., 9, 11, and 16a; group e., 11b and 20; and group f., 21 and 23. The average rate of growth of the above groups is shown in Figure 3.

TABLE 5—*Size in millimeters of colony of Fusarium isolants at various temperatures on ammonium nitrate medium, at the end of the seventh day*

	Diameter of colonies in mm. at various temperatures				
Isolant	15°	20°	25°	30°	35° C.
1	20	32	60	75	10
2	22	32	55	65	13
3	20	32	55	65	10
4	20	32	52	62	11
5	20	32	57	65	10
6	22	35	55	67	10
7	12	25	34	42	11
7b*	13	22	33	40	12
7c	15	20	32	30	13
8	20	33	57	55	12
9	15	40	73	77	13
10	10	15	30	35	23
11	21	40	65	80	12
11b	20	27	40	40	12
11c	19	45	50	55	10
12	15	22	35	35	17
13	14	15	30	35	23
14	13	20	32	35	25
15	12	17	30	35	20
16	20	30	60	60	13
16a	25	40	75	85	10
16b	20	28	50	55	13
17	13	20	32	30	20
18	12	17	25	30	21
19	15	24	35	47	25
20	16	38	40	45	12
21	23	47	72	70	13
22	12	22	34	40	25
22b	12	22	35	40	21
23	30	45	65	70	9

* Letters a, b, c indicate dissociant of corresponding isolant.

No consistent correlation was found between the maximum amount of growth in artificial culture at 30° C. and virulence, for virulent strains were to be found in each of the above groups. For example, the

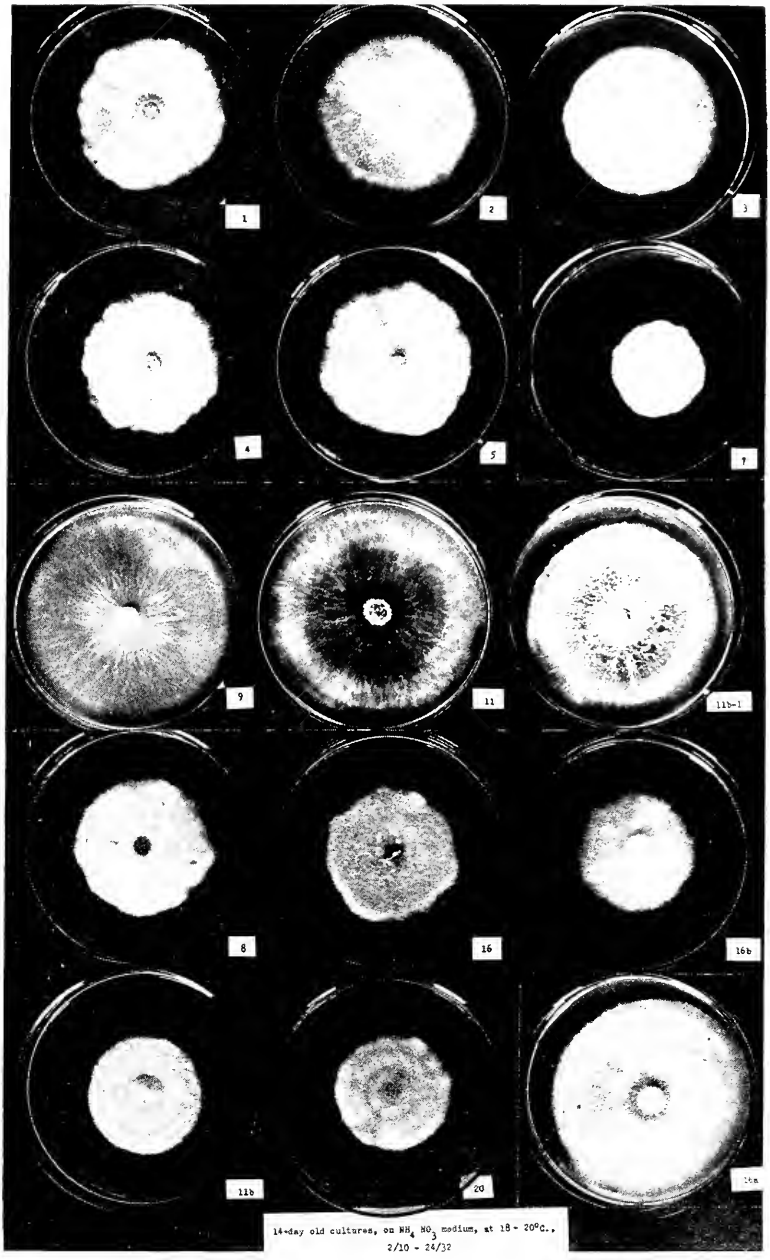

FIG. 2—Cultures of various strains of *Fusarium niveum*, 14 days old and grown under like conditions. Note variations in growth and type of mycelium

highly-virulent strains 3, 11, and 20 are found in separate groups. Of these, strain 20 is the more virulent and the slowest grower. On the other hand, slightly pathogenic or non-virulent strains are found distributed among the six groups, with the greater number in group b.

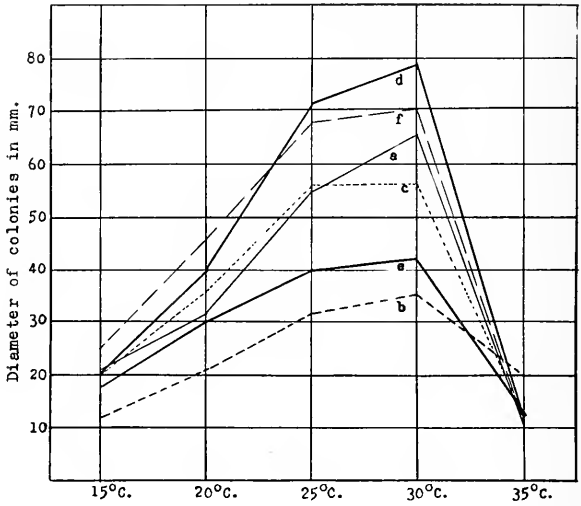

FIG. 3—Average growth rate of six groups of isolants at the end of 7 days, on the same substrate, grown at different temperatures

HYDROGEN-ION CHANGES IN CULTURE

It has been observed[11,24] that certain species of fungi grown in nutrient solution either increase or decrease the hydrogen-ion concentration of the medium. An experiment was set up to determine whether the various isolants of *Fusarium niveum* would produce any significant differential change in the pH of the culture solution.

Flasks of 50 cc. capacity containing 40 cc. of ammonium nitrate liquid medium (pH 4.4) were inoculated with an 8 mm. disk cut from the margin of petri-dish cultures of isolants 5, 8, 20, and 23. The cultures and checks were incubated at 28° C. At three-day intervals, two cultures of each isolant and check solutions were tested by the colorimetric method for determining pH values. Results of these tests are presented in Table 6 and Figure 4.

When tested on the third day after inoculation, the acidity of the four cultures had increased from pH 4.4 to pH 3.8 or slightly less. After the third day the cultures tended to become less acid until a maximum alkalinity, around pH 7.8, was reached for isolants 5, 20, and 23 on the

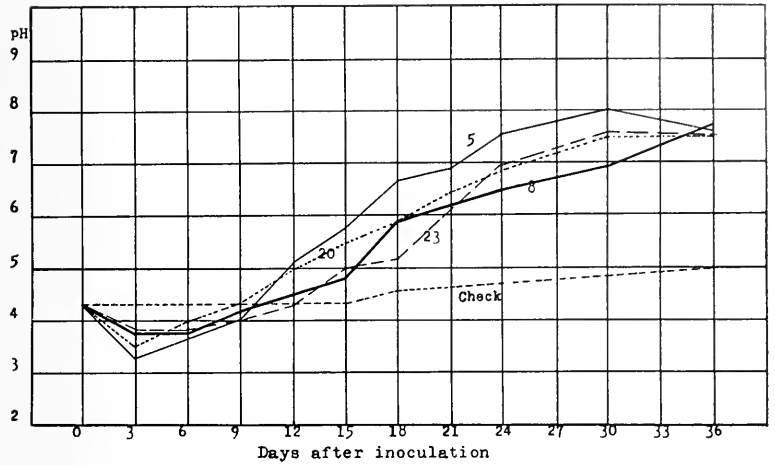

FIG. 4—Changes in the pH of ammonium nitrate nutrient solution, by isolants 5, 8, 20, and 23 at 28° C. (Taken from data in Table 6)

30th day, and for isolant 8 on the 36th day. There was a decrease of acidity in the checks from an initial pH 4.4 to 5.0 at the end of 36 days.

The difference in pH values of the four isolants at any one period after inoculation was not sufficient to distinguish one isolant from another with any degree of certainty. Apparently by the end of 36 days a pH equilibrium of 7.5 to 7.8 was reached for the four strains. It is interesting to note that young cultures of isolants 5, 8, 20, and 23, under the conditions of the experiment, tended first to increase the acidity of the culture solution and later to decrease it with time. This condition is most likely explained on the basis that the ammonia from the ammonium nitrate is first utilized, causing a decrease in pH; then when the NO_3 ion becomes more readily utilized than the NH_4 ion the pH goes up. At least this is characteristic of higher plants.

TABLE 6—*Changes in pH produced by four Fusarium niveum isolants in ammonium nitrate nutrient solution at 28° C. over a 36-day period*

Number days after inoculation	Sterile solution	pH readings			
		Strains			
		5	8	20	23
0	4.4	4.4	4.4	4.4	4.4
3	4.4	3.4	3.8	3.6	3.8
6	4.4	3.6	3.8	4.0	3.8
9	4.4	4.0	4.2	4.3	4.0
12	4.4	5.1	4.5	5.0	4.4
15	4.4	5.8	4.8	5.5	5.0
18	4.6	6.7	5.9	5.9	5.2
21	4.6	6.9	6.2	6.4	6.1
24	4.6	7.5	6.4	6.8	6.9
27	no test				
30	4.8	8.0	6.9	7.5	7.6
33	no test				
36	5.0	7.6	7.8	7.5	7.5

DISSOCIATION

The appearance of different types of growth on artificial medium from single spore cultures has been observed by many investigators and called by a variety of names. The term "dissociation" as interpreted by Leonian[9] will be used in this paper when referring to this phenomenon.

Leonian[9,10] believes dissociation to be "that phenomenon whereby a given organism traces the sphere of variability of the species." In species where dissociation occurs, all the dissociants fall within the limits of the species. Conversely, the limits of the species are determined by the number and diversity of the dissociants. Certain bonds or common characteristics must be recognized in order properly to classify dissociants or strains which have occurred in nature.

The failure of investigators to recognize the existence of dissociation within a species has brought about much confusion, and as a result there are today many species which deserve no rank higher than that of variety or strain. However, the difficulty of recognizing the true relationship between isolants obtained from diverse sources makes the recognition of dissociants under natural conditions not so simple. From the investigator's viewpoint the most reliable evidence of the relationship between dissociants or strains, sometimes called forms or biotypes, is that of actually observing a distinct, new, and diverse type sectoring out in pure culture. Often the relationship of strains, forms, or varieties which have occurred in nature and are described as species can be traced by comparison with known dissociants of the species in question. By a careful study of the morphological and physiological characters[9,10] one will find a certain specific line of behavior present throughout all strains and variants of a given species of fungus. It is to be regretted that no standardized system of such tests has been devised and accepted by biological workers in this field.

The appearance of dissociants in culture was frequently observed by the writer. Only a few of the more distinct ones were continued as subcultures of the original isolant. Two dissociants, 7b and 7c from isolant 7, shortly reverted to types similar to the original isolant. Dissociants 11a and 11b of isolant 11 have shown a marked degree of stability, although 11b gave rise to dissociant 11b-1, which closely resembles the parental form 11. Two dissociants, 16a and 16b, from isolant 16 were constant in their behavior over a period of 9 months. (Fig. 5.) Tests have shown that these dissociants of 11 and 16 differ from the parental strain in their physiological and pathological behavior (see Table 5).

Since dissociants appeared in artificial culture, it seemed reasonable to suppose that they would occur as readily in the soil. In order to check this assumption 50 to 60 plants from each flat in series IV, irrespective of wilting, were plated out. As was to be expected, the fungus was recovered from the wilted plants and also in many instances from apparently healthy plants.

The general method of isolating the fungus from the melon seed-

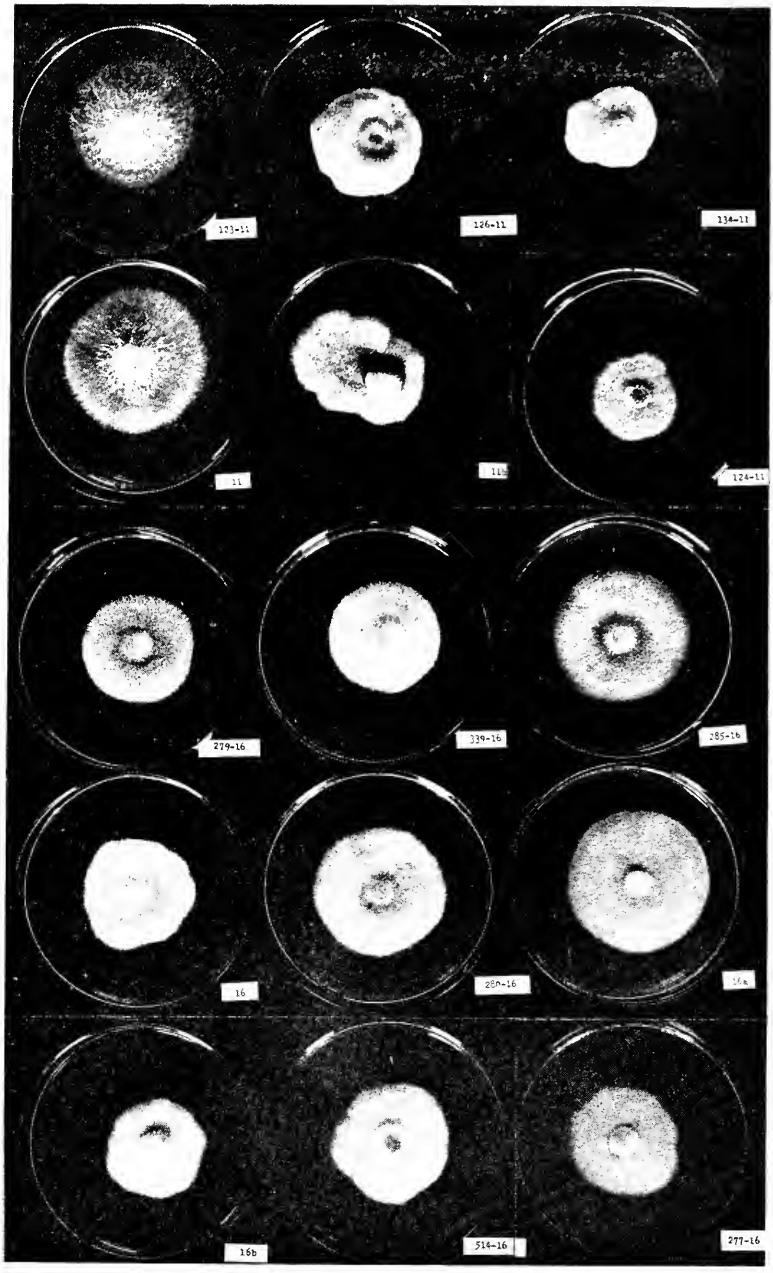

FIG. 5—Strains 11 and 16, artificial culture dissociants 11b, 16a, and 16b, and isolants recovered from inoculated soil, including parental types and dissociants

lings was modified in this particular case in order to reduce to a minimum any surface contamination of the seedlings. After careful washing in running tap water the seedlings were placed in 95% alcohol for 2 to 3 minutes, removed to a 1:1000 solution of bichloride of mercury, where they were left for 8 to 10 minutes and, in a few instances, longer, then rinsed in sterile distilled water, and finally plated on ammonium nitrate agar plates.

TABLE 7—*Description of dissociants from flats inoculated with isolants 11 and 16 of Fusarium niveum*

Original strain	Identification number	Number of times isolated	Description
11	123-11	4	Like strain 11
	124-11	35	Like dissociant 11b, which appeared in artificial culture.
	134-11	2	Intermediate between 11b and strain 20.
	126-11	1	Similar to 11b, with more abundant mycelium.
	16-11	24	Same as strain 7.
16	280-16	2	Like strain 16.
	339-16	1	Like strain 16.
	514-16	1	Intermediate between 16 and 16b.
	279-16	1	Similar to 16, with less dense and radiating mycelium.
	285-16	4	Same as dissociant 16a, which appeared in artificial culture.
	277-16	40	Same as dissociant 16b, which appeared in artificial culture.
	505-16	15	Same as strain 7.

In addition to the original strain used to inoculate the flats, a number of variants were isolated as well. Some of the isolants resembled strains recovered from other flats. These results indicated that a change must have occurred in the various strains while in the soil, or that the flats were contaminated. The former condition is believed to have taken place, since the check flats were not contaminated, and since in three specific instances the variants from the soil were identical with the dissociants which occurred in artificial culture. In other cases the variants recovered from the soil resembled the original type, but at the same time showed minor but distinct variations.

A number of diverse isolants were recovered from flats 11 and 16. In addition to the original types, isolants similar to the dissociants which had appeared in artificial culture from strains 11 and 16 were also recovered. The number of dissociants isolated from these flats is given in Table 7 and illustrated in Figure 5.

A soil dissociant, 124-11,* isolated from a number of plants, resembled the dissociant 11b that had appeared in artificial culture. A second soil dissociant, 134-11, appeared to be an intermediate form between 11b and isolant 20. Another had many of the characteristics of 11b with an increase in mycelial growth. In addition to these variants, which showed unmistakable evidence of their relationship to the original

* The joint numbers refer to isolants from plants grown in flats inoculated with the strain designated by the second half of the number.

isolant and to the dissociant in artificial cultures, several recovered variants resembled strain 7.

A number of the isolants recovered from flat 16 resembled the original strain 16, and some were like its dissociants. The isolants 280-16 and 339-16 were the same as strain 16, while 279-16 had a less dense and more radiating growth. A large number of isolations of the soil isolants, 277-16, were recovered which were identical with 16b, which had appeared in artificial culture. One isolant, 515-16, appeared to be intermediate between 16 and 16b. Four of the isolants were like 16a, and another like the dissociant in artificial cultures. In addition to these closely-related soil dissociants from flat 16, a number were recovered which were like strain 7.

The foregoing evidence indicates that dissociation occurs in the soil, perhaps as readily as in artificial culture. The number of like dissociants which have occurred in a single flat is considered as evidence that the variation in the organism must have occurred in the soil rather than in the plant or upon the culture medium in the process of isolation and identification.

DISCUSSION

A study of the data presented shows that physiologic specialization exists within the species *Fusarium niveum*. Both pathogenic and cultural tests have shown distinct differences in the 23 isolants studied. The isolants range from apparently non-pathogenic to highly virulent strains under the conditions of the greenhouse tests. In pure culture distinct differences also were found in many of the isolants. These variations in virulence and the rate of growth are sufficient to designate at least a majority of the 23 isolants as distinct strains, of which 1, 2, 3, 5, 7, 8, 9, 10, 11, 16, and 23 proved to be the most distinctive. However, the writer makes no attempt to classify any given isolant as a permanent strain.

The establishment of strains of the fungus studied is complicated by dissociation, a phenomenon common in most fungi. Because of this the stability of any single isolant or strain cannot be considered permanently dependable. Suddenly and for no apparent reason, changes may take place in the morphology, physiology, or pathogenicity of an apparently stable strain. Consequently the establishment and identification of strains is not feasible, except for the duration of the period of study. Nevertheless, this does not lessen the fact that in the field one may pick up isolants with varying degrees of virulence or possessing other variable characters, such as rate and type of growth in artificial culture.

Any attempt to establish a permanent strain of a Fusarium species is likely to meet with disappointment, for the permanency of a strain or isolant may continue for several years or it may be short-lived. The latter condition is likely to be the more prevalent. Leonian[10] found that isolants of *Fusarium moniliforme* were highly inconstant in their

ability to bring about infection, and since the morphologic and physiologic characters of this fungus are governed by an extreme plasticity, he sees no reason why pathogenicity should be an exception to the rule. This inconstancy of isolants is by no means confined to *F. moniliforme,* for similar conditions, perhaps not so pronounced, have been observed in *Fusarium niveum* and other fungi.

The possibility that dissociation occurs in the soil, thereby giving rise to new or different strains, has been overlooked by many investigators. In two instances isolants were recovered from plants growing in infested soil which were identical with dissociants which had previously appeared in artificial culture. This phase of dissociation, which has been suspected by a few investigators, explains why isolants from diverse localities or even from the same locality may vary in pathogenicity or other characteristics. The writer is of the opinion that dissociation occurring in the soil is responsible for the appearance of many variable isolants or strains.

Since dissociation in the soil must be of frequent occurrence, and the direction which the variation may take, a matter of chance, the number of distinct strains (degrees of physiologic specialization) appears to be infinite. There is always the possibility that more virulent strains may come into existence, or that the dissociant may acquire the ability to attack a new host. Thus a given organism may be a potential progenitor of a number of strains with diverse host relationships. This latter condition is suggested by the morphological similarity of several of the vascular Fusaria, which differ materially only in their host relationship.

The apparent breakdown in the resistance of the Conqueror watermelon when introduced into a new watermelon-growing section, or after a period of years, can readily be explained on the basis of physiologic specialization on the part of the pathogen. With the possibility that new or more virulent strains of the pathogen may arise, the dissociation phenomenon will ever be a Damoclean threat to the plant breeder. Therefore any so-called resistant variety should be tested against a large number of pathogenic strains, in order to test the chances of its survival.

We have reasons to believe that a more exhaustive study will bring to light strains which will gradate from non-pathogenic to extremely virulent strains and differ as well in other characteristics.

SUMMARY AND CONCLUSIONS

Comparative pathological and cultural tests were made with 23 isolants of *Fusarium niveum* obtained from widely separated localities.

The isolants showed different degrees of virulence toward watermelon seedlings grown in greenhouse tests. Strains 3, 11, and 20 were found to be highly virulent, strains 1, 4, 6, 8, 16, and 23 slightly less virulent, strains 2, 5, and 7 moderately virulent, and the remaining eleven strains only slightly virulent or non-virulent upon watermelon seedlings in these tests.

The pathogenicity of these strains varied with the resistance of the suscept, the seedlings of watermelon varieties bred and selected for wilt resistance being more resistant than the common commercial sorts. All varieties tested were found to be susceptible to the more virulent strains of *Fusarium niveum*.

The optimum temperature for growth of the fungus in culture was found to lie between 24° and 28° C., with the maximum slightly above 35° C. and the minimum about 5° C.

No correlation was observed between virulency and rate of growth in artificial culture.

When strains 5, 8, 20, and 23 were grown on a so-called ammonium-nitrate solution with an initial pH of 4.4, the acidity increased to a pH of 3.8 or slightly below this point in 72 hours, after which the acidity decreased to a pH value within the limits of 7.5 to 7.8 at the end of 36 days.

Dissociants of strains 11 and 16 appeared in artificial culture; similar dissociants were recovered from plants growing in flats inoculated with these strains. In two cases these were identical with dissociants obtained in artificial culture. This behavior of the fungus in the soil indicates that dissociation is not confined to an artificial medium. The dissociant from one strain often resembled that of another or one of the original isolants.

The apparent breakdown in the resistance of a given variety to a pathogen may be explained on the basis of new and more virulent strains which dissociate from less virulent or non-pathogenic strains.

It seems likely that the development of a resistant watermelon must depend upon its resistance not only to one but to many pathogenic strains. In testing the resistance of a variety it is obvious that many pathogenic strains of the pathogen should be used.

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